IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	:	
Prasad DEVARAJAN et al.	:	Confirmation No: 2792
Serial No.: 10/811,130	:	Group Art Unit: 1641
Filed: March 26, 2004	:	Examiner: FOSTER, Christine E.

A METHOD AND KIT FOR DETECTING THE EARLY ONSET OF RENAL TUBULAR CELL INJURY

DECLARATION UNDER 37 CFR 1.131

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

I, Prasad Devarajan, do hereby declare as follows:

1. I am a co-inventor of the subject matter described and claimed in the above-identified patent application.

2. I hold an undergraduate degree in Biology and a Medical Doctor (M.D.) degree from Bombay University, India. I also completed graduate research in renal disease in the Department of Physiology, and a residency in Pediatrics at SUNY at Stony Brook, NY. I also completed a fellowship in Nephrology at Yale University, and completed an NIH-sponsored research fellowship in renal disease at Yale.

3. I have been conducting research in the field of renal disease since 1985. I am presently the Louise M. Williams Endowed Chair, Professor of Pediatrics, Professor of Developmental Biology, Director of Nephrology and Hypertension, Director of Nephrology

Clinical Laboratories, CEO of Dialysis Unit, at Cincinnati Children's Hospital Medical Center and the University of Cincinnati School of Medicine, Cincinnati, OH.

I am an expert reviewer of grant applications in the field of renal diseases for the NIH and several other national and international organizations. I am an expert reviewer of publications submitted to more than 20 scientific and medical journals in the field of renal disease. I am a member of the Editorial Board of key journals in the field of renal disease. I am on the Advisory Board and Research Committees of the American Society of Nephrology, American Society of Pediatric Nephrology, International Acute Kidney Injury Network, and the National Institutes of Health in the field of renal disease.

4. I have read and am familiar with the presently-amended claims of the above-identified patent application, and have read and am familiar with the publication of Muramatsu et al. (November, 2002).

5. Prior to November 1, 2002, experiments were conducted in my laboratory, at my instruction and direction, involving the detection of a 25 kDa protein, identified as NGAL, in the urine of a mouse following an acute ischemic-reperfusion injury (IRI) that resulted in acute renal failure (ARF).

6. Attached Exhibit A provides true copies of the cover and of pages 45, 48 and 49 from a notebook that is maintained in my laboratory and under my control, on which is recorded an experiment described below. Attached Exhibit B is a true copy of a photograph of western blots performed during the experiment, with hand-written markings and notations contemporaneously written onto the film. In the attached true copies of the above pages and film photograph, all dates and unrelated information have been redacted. All such dates are prior to November 1, 2002.

7. Notebook page 45, in the middle of the page, describes an experiment for Ischemic Reperfusion Injury (IRI) in the kidneys of mice. A surgical procedure or operation was

performed on mice where IRI was induced by clamping both kidney arteries ["kidney's pedicle (two kidneys)"] for 30 minutes, and the urine was collected before and after the operation at 1 hr, 2 hr, 3 hr, 4 hr and 5 hr. The bottom of page 45 describes the fate of the four mice on which the operation was performed. Mice #2 [②] and #3 [③] survived and where alive after the procedure, and urine samples and blood samples of mice #2 and #3 were taken and assayed as described on pages 48 and 49.

8. Notebooks page 48 and 49 of Exhibit A describe assaying of the urine samples obtained from mice #2 and #3 by western blot with a primary antibody for lipocalin (identified as NGAL). Exhibit B is a copy of a photograph of the urine assay results by western blot for mice #2 and #3, showing no or nominal NGAL at time 0, and elevated quantities of NGAL that increased with increasing time after the surgical procedure.

9. While the copy is not as clear as the photograph or the original gels, Exhibit B states in conclusion "urine NGAL detected early 2-3 hr after IRI".

10. Notebook page 49 (middle of the page) identifies an increased level of plasma creatinine in mice #2 and #3 at 24 hours (after the event), establishing the onset or presence of acute renal failure (ARF).

11. In my opinion, this experiment shows the conception and reduction to practice of a method for determining if a mammal has an acute ischemic renal tubular cell injury that can progress to ARF. The experiment provided a mammal on which a surgical procedure, as an event, was performed. The surgical procedure was suspected of causing an acute ischemic renal tubular cell injury that predisposed the mammal to progressing to ARF. The experiment included obtaining urine samples from the mammal within a period of time of 12 hours after the event, and described western blot assays to identify the quantities of lipocalin (NGAL) protein in the obtained urine samples. The experiment also showed that the elevated quantities of NGAL correlated with the later progression of the mice to ARF at 24 hours, as evidenced by the elevated levels of plasma creatinine at 24 hours.

12. Shortly after I obtained the results of this experiment, I initiated steps to contact legal counsel on the patentability of the invention.

I further declare that all statements made of my knowledge are true and that all statements made on information and belief are believed to be true; further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under 18 USC 1001 and may jeopardize the validity of the application or any patent issuing thereon.

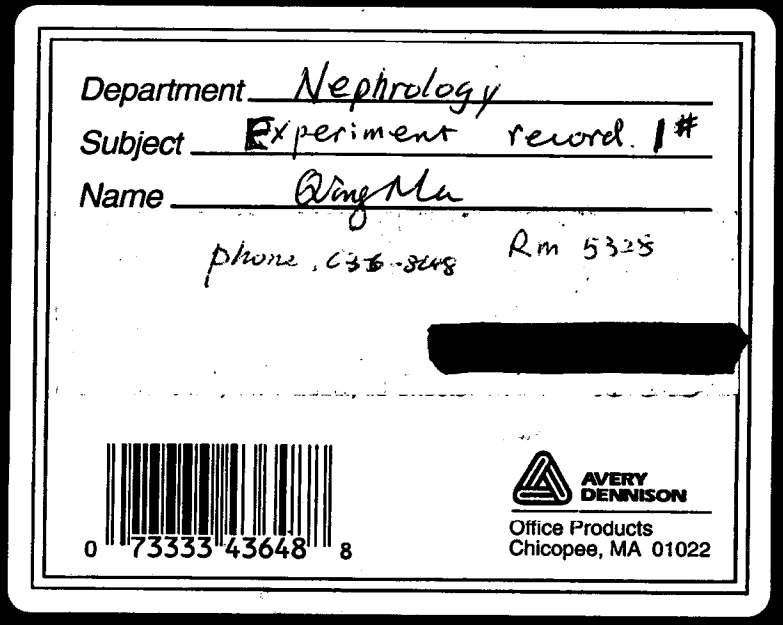
05/29/2008
Date

Prasad Devarajan

18 USC 1001: "Whoever in any matter within the jurisdiction of any department or agency of the United States knowingly and willfully falsifies, conceals or covers up by any trick, scheme, or device a material fact, or makes any false, fictitious or fraudulent statements or representations, or makes or uses any false writing or document knowing the same to contain any false, fictitious or fraudulent statement or entry, shall be fined not more than $10,000 or imprisoned not more than five years, or both."

Exhibit A (4 pages)

Declaration under 37 CFR 1.132 of Prasad Devarajan
US Patent Application 10/811,130



[redacted]

Experiment for kidney of mice IRI. Ann prader show

- Prepare for surgery.

Operate on mouse's abdomen. Cut 2-2.5cm incision, expose kidney.

Clamp kidney's pedicle. (two kidney) for 30 min.

release clamps.

Sew the wounds.

- Let mice recover at mice cadge. with warm condition.

- Collect urine.

before surgery overnight

after surgery 1hr 2hr 3hr 4hr 5hr

① mouse died during surgery → take blood, kidney

② alive

③ alive

④ After surgery 1 hour, mouse died → take blood kidney

DRL

Western Blotting for urine of mice. [redacted]

Sample: ② o/n, [redacted] 2hr 3hr 4hr 5hr o/n 2 [redacted]

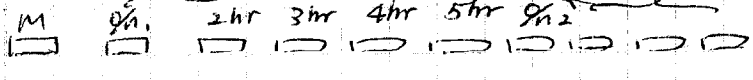
③ o/n, [redacted] 3hr 4hr 5hr o/n 2 [redacted]

Prepare Sample:

- Take Sample from 4°c.
- Centrifuge 14.5 X1000 rpm. 5min. RT. Balance !!
- Take Supernatant, put them in new tubes.
- Put 25 ul urine at small new tubes.
- Add 25 ul Laemmli Sample buffer (2x).
- Boiling Sample., 10 min
- Loading.

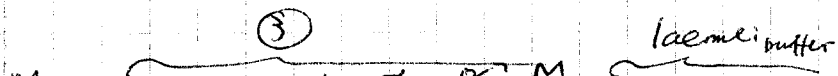
Make 12% Gel

Gel, ② laemmli buffer



50ul/well

Gel₂ ③ laemmi buffer



50ul/well

- Run 12% SDS Gel, 200V, about 1 hour.
- Transfer membrane. 40mA overnigh. at 4°c.

[redacted]

Finish Western Blotting for urine of mice.
5% milk block membrane for 30 min

Incubate 1° Ab Lipocalin 1:500 (the fifth time) for 1 hour.

Wash membrane for 30 min

Incubate 2° Ab goat anti Rabbit 1:2000 (the fifth time) for 1hour
Wash 30min
ECL incubate 1min
Develop film.

We get ugly band. the bands deform.

Autodave tubes

Chang Reptee cells media

Aliquot urine sample + 2x Samp buffer

Creatinine (mg/dl)
24hr plasma

2. 0.840 mg/dl

3 6.62 mg/dl

Control — 1hr = 0.74mg/dl (no clamped)
4 mice — 2hr death = 5.72 mg/dl (clamped)

[redacted]

- Mix immediately and thoroughly on a titerplate shaker
allow to ~~stand~~ 5min at RT.
2f PDT is dissolves after mixing

- Read and record Absorbance (A) of standard at test vs Blank
[this is Final A]
These can be put in duplicates when reading at Eliza plate Reader.

Calculation of Creatinine concentration

$$\text{Creatinine (mg/dl)} = \frac{\text{INITIAL Test} - \text{FINAL Test}}{\text{INITIAL standard} - \text{FINAL standard}} \times 3^{\#}$$

Exhibit B (1 page)

Declaration under 37 CFR 1.132 of Prasad Devarajan - US Patent Application 10/811,130